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Calcium rubies: A family of red-emitting functionalizable indicators suitable for two-photon Ca2+ imaging

Collot M., Loukou C., Yakovlev A., Wilms C., Li D., Evrard A., Zamaleeva A., Bourdieu L., Léger J., Ropert N., Eilers J., Oheim M., Feltz A., Mallet J.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

We designed Calcium Rubies, a family of functionalizable BAPTA-based red-fluorescent calcium (Ca2+) indicators as new tools for biological Ca2+ imaging. The specificity of this Ca2+-indicator family is its side arm, attached on the ethylene glycol bridge that allows coupling the indicator to various groups while leaving open the possibility of aromatic substitutions on the BAPTA core for tuning the Ca2+-binding affinity. Using this possibility we now synthesize and characterize three different CaRubies with affinities between 3 and 22 μM. Their long excitation and emission wavelengths (peaks at 586/604 nm) allow their use in otherwise challenging multicolor experiments, e.g., when combining Ca2+ uncaging or optogenetic stimulation with Ca2+ imaging in cells expressing fluorescent proteins. We illustrate this capacity by the detection of Ca2+ transients evoked by blue light in cultured astrocytes expressing CatCh, a light-sensitive Ca2+-translocating channelrhodopsin linked to yellow fluorescent protein. Using time-correlated single-photon counting, we measured fluorescence lifetimes for all CaRubies and demonstrate a 10-fold increase in the average lifetime upon Ca2+ chelation. Since only the fluorescence quantum yield but not the absorbance of the CaRubies is Ca2+-dependent, calibrated two-photon fluorescence excitation measurements of absolute Ca2+ concentrations are feasible.

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